

|    | L # | Hits       | Search Text   | DBs                | Time Stamp          |
|----|-----|------------|---|--------------------|---------------------|
| 1  | L3  | 14987      | biolumines\$ or fluorescen\$ near4 protein\$1 or luciferase\$1 or photoprotein\$1 | USPAT;<br>US-PGPUB | 2003/02/24<br>15:09 |
| 2  | L4  | 13461<br>5 | bubble\$  | USPAT,<br>US-PGPUB | 2003/02/24<br>15:10 |
| 3  | L5  | 878        | 3 and 4   | USPAT,<br>US-PGPUB | 2003/02/24<br>15:10 |
| 4  | L6  | 15         | 3 same 4  | USPAT,<br>US-PGPUB | 2003/02/24<br>17:01 |
| 5  | L7  | 68310      | toy or novelty  | USPAT,<br>US-PGPUB | 2003/02/24<br>15:40 |
| 6  | L8  | 30         | 5 and 7   | USPAT,<br>US-PGPUB | 2003/02/24<br>15:40 |
| 7  | L9  | 17         | 3 same 7  | USPAT;<br>US-PGPUB | 2003/02/24<br>17:01 |
| 8  | L10 | 20         | 3 and toy   | USPAT;<br>US-PGPUB | 2003/02/24<br>17:09 |
| 9  | L11 | 13         | 10 not 8  | USPAT,<br>US-PGPUB | 2003/02/24<br>17:25 |
| 10 | L12 | 22         | 3 and novelty adj item\$1   | USPAT;<br>US-PGPUB | 2003/02/24<br>17:24 |
| 11 | L13 | 12         | 12 not 8  | USPAT;<br>US-PGPUB | 2003/02/24<br>17:25 |

PGPUB-DOCUMENT-NUMBER: 20030013103

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030013103 A1

TITLE: Apparatus and method for detecting and identifying infectious agents

PUBLICATION-DATE: January 16, 2003

US-CL-CURRENT: 435/6,356/319 ,435/287.2 ,435/7.9

APPL-NO: 10/ 126139

DATE FILED: April 19, 2002

RELATED-US-APPL-DATA:

child 10126139 A1 20020419 parent division-of 08990103 19971212 US GRANTED  
parent-patent 6458547 US non-provisional-of-provisional 60037675 19970211 US  
non-provisional-of-provisional 60033745 19961212 US

#### RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn.119(e) to U.S. Provisional application Serial No. 60/037,675, filed Feb. 11, 1997 and to U.S. Provisional application Serial No. 60/033,745, filed Dec. 12, 1996. [0002] Certain subject matter in this application is related to subject matter in U.S. application Ser. No. 08/757,046, filed Nov. 25, 1996, to Bruce Bryan entitled "BIOLUMINESCENT NOVELTY ITEMS" (B), and to U.S. application Ser. No. 08/597,274, filed Feb. 6, 1996, to Bruce Bryan, entitled "BIOLUMINESCENT NOVELTY ITEMS". This application is also related to U.S. application Ser. No. 08/908,909, filed Aug. 8, 1997, to Bruce Bryan entitled "DETECTION AND VISUALIZATION OF NEOPLASMS AND OTHER TISSUES" and to U.S. Provisional application Serial No. 60/023,374, filed Aug. 8, 1996, entitled "DETECTION AND VISUALIZATION OF NEOPLASMS AND OTHER TISSUES", and also to published International PCT application No. WO 97/. [0003] The subject matter of each of the above noted U.S. applications, provisional applications and International application is herein incorporated by reference in its entirety.

PGPUB-DOCUMENT-NUMBER: 20020132318

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020132318 A1

TITLE: Fluorescent proteins

PUBLICATION-DATE: September 19, 2002

US-CL-CURRENT: 435/183,435/320.1 ,435/325 ,435/69.1 ,530/350 ,536/23.2

APPL-NO: 10/ 060857

DATE FILED: January 29, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60264932 20010129 US

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn. 119(e) from provisional application 60/264,932 filed Jan. 29, 2001. The contents of this application are incorporated herein by reference.

PGPUB-DOCUMENT-NUMBER: 20020090659

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020090659 A1

TITLE: Detection and visualization of neoplastic tissues and other tissues

PUBLICATION-DATE: July 11, 2002

US-CL-CURRENT: 435/7.23,424/9.6

APPL-NO: 09/746485

DATE FILED: December 22, 2000

RELATED-US-APPL-DATA:

child 09746485 A1 20001222 parent continuation-of 08908909 19970808 US UNKNOWN

#### RELATED APPLICATIONS

[0001] This application is a continuation of allowed U.S. application Ser. No. 08/908,909, filed Aug. 8, 1997 to Bruce Bryan, entitled "DETECTION AND VISUALIZATION OF NEOPLASTIC TISSUES AND OTHER TISSUES." This application and U.S. application Ser. No. 08/908,909 claim the benefit of priority under 35 U.S.C. § 119(e) to U.S. provisional application Ser. No. 60/023,374 to Bruce Bryan, filed Aug. 8, 1996, and entitled DETECTION AND VISUALIZATION OF NEOPLASMS AND OTHER TISSUES. [0002] Subject matter in this application is related to subject matter in allowed U.S. application Ser. No. 08/597,274 to Bruce Bryan, filed Feb. 6, 1996, entitled "BIOLUMINESCENT NOVELTY ITEMS", and U.S. application Ser. No. 08/757,046 to Bruce Bryan, filed Nov. 25, 1996, now U.S. Pat. No. 5,876,995, entitled "BIOLUMINESCENT NOVELTY ITEMS". The subject matter of each of U.S. application Ser. No. 08/597,274 and U.S. application Ser. No. 08/757,046, and U.S. provisional application Ser. No. 60/023,374 is herein incorporated in its entirety by reference thereto.

PGPUB-DOCUMENT-NUMBER: 20010036073

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010036073 A1

TITLE: Carvable decorative gourd

PUBLICATION-DATE: November 1, 2001

US-CL-CURRENT: 362/154,362/122 ,362/124 ,362/808

APPL-NO: 09/ 838616

DATE FILED: April 19, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60198429 20000419 US

RELATED APPLICATIONS

[0001] This application is a continuation-in-part application of the provisional application entitled "Carvable Decorative Gourd", Serial No. 60/1908,429, filed Apr. 19, 2000,

US-PAT-NO: 6416960

DOCUMENT-IDENTIFIER: US 6416960 B1

TITLE: Detection and visualization of neoplastic tissues and other tissues

DATE-ISSUED: July 9, 2002

US-CL-CURRENT: 435/7.23; 424/130.1 ; 424/133.1 ; 424/138.1 ; 424/141.1

APPL-NO: 08/ 908909

DATE FILED: August 8, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application claims the benefit of priority under 35 U.S.C. .sctn.119(e) to U S provisional application Ser. No. 60/023,374 to Bruce Bryan, filed Aug. 8, 1996, and entitled DETECTION AND VISUALIZATION OF NEOPLASMS AND OTHER TISSUES.

US-PAT-NO: 6126870  
DOCUMENT-IDENTIFIER: US 6126870 A

TITLE: Chemiluminescent labeling compounds

DATE-ISSUED: October 3, 2000

US-CL-CURRENT: 252/700; 435/4 ; 435/5 ; 435/6 ; 435/7.1 ; 544/212 ; 544/96  
; 546/102 ; 546/103 ; 546/104

APPL-NO: 09/ 099657

DATE FILED: June 17, 1998

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION This application is a  
continuation-in-part of applicants' co-pending U.S. application Ser. No.  
08/927,381 filed on Sep. 12, 1997.

US-PAT-NO: 6017769

DOCUMENT-IDENTIFIER: US 6017769 A

TITLE: Non-enzymatic methods of generating chemiluminescence from acridan alkenes

DATE-ISSUED: January 25, 2000

US-CL-CURRENT: 436/544; 435/26 ; 435/28 ; 435/6 ; 435/7.1 ; 435/968 ; 436/546  
; 436/800 ; 436/805

APPL-NO: 09/ 099656

DATE FILED: June 17, 1998



US-PAT-NO: 5931383  
DOCUMENT-IDENTIFIER: US 5931383 A

TITLE: Self-illuminated drinking straw

DATE-ISSUED: August 3, 1999

US-CL-CURRENT: 239/33

APPL-NO: 09/ 017992

DATE FILED: February 3, 1998

US-PAT-NO: 5858693

DOCUMENT-IDENTIFIER: US 5858693 A

TITLE: Device and method for phage-based antibiotic susceptibility testing

DATE-ISSUED: January 12, 1999

US-CL-CURRENT: 435/8; 435/32 ; 435/4 ; 435/5 ; 436/149 ; 436/63

APPL-NO: 08/ 883722

DATE FILED: June 27, 1997

PARENT-CASE:

This is a division of application Ser. No. 08/480,807, filed Jun. 7, 1995 now abandoned.

US-PAT-NO: 5840963

DOCUMENT-IDENTIFIER: US 5840963 A

TITLE: Chemiluminescent reactions using dihydroxyaromatic compounds and heterocyclic enol phosphates

DATE-ISSUED: November 24, 1998

US-CL-CURRENT: 562/23; 562/25

APPL-NO: / 021322

DATE FILED: February 10, 1998

PARENT-CASE:

This application is a divisional application of copending allowed application Ser. No. 08/855,421, May 13, 1997.

US-PAT-NO: 5772926

DOCUMENT-IDENTIFIER: US 5772926 A

TITLE: Chemiluminescent reactions using dihydroxyaromatic compounds and heterocyclic enol phosphates

DATE-ISSUED: June 30, 1998

US-CL-CURRENT: 252/700; 435/4

APPL-NO: 08/ 855421

DATE FILED: May 13, 1997

US-PAT-NO: 5554035

DOCUMENT-IDENTIFIER: US 5554035 A

TITLE: Bioluminescent algae in light bulb shaped viewing device

DATE-ISSUED: September 10, 1996

US-CL-CURRENT: 434/297; 119/245

APPL-NO: 08/ 269696

DATE FILED: July 1, 1994

PGPUB-DOCUMENT-NUMBER: 20020132318

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020132318 A1

TITLE: **Fluorescent proteins**

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

| NAME        | CITY      | STATE | COUNTRY | RULE-47 |
|-------------|-----------|-------|---------|---------|
| Zhao, Ming  | San Diego | CA    | US      |         |
| Xu, Mingxu  | La Jolla  | CA    | US      |         |
| Jiang, Ping | San Diego | CA    | US      |         |
| Yang, Meng  | San Diego | CA    | US      |         |

APPL-NQ: 10/ 060857

DATE FILED: January 29, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60264932 20010129 US

US-CL-CURRENT: 435/183,435/320.1 ,435/325 ,435/69.1 ,530/350 ,536/23.2

ABSTRACT:

Improved forms of **fluorescent protein with high fluorescence** and low toxicity are disclosed.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. .sctn. 119(e) from provisional application 60/264,932 filed Jan. 29, 2001. The contents of this application are incorporated herein by reference.

----- KWIC -----

Title - TTL:

Fluorescent proteins

Abstract Paragraph - ABTX:

are disclosed.

Summary of Invention Paragraph - BSTX:

[0002] The invention relates to new forms of "green **fluorescent protein**" and their uses. Specifically, the invention is directed to a particular GFP and variants thereof which are bright and nontoxic.

Summary of Invention Paragraph - BSTX:

[0003] Green **fluorescent protein** (GFP) was initially isolated from *Aequorea victoria* by Chalfie, U.S. Pat. No. 5,491,084. Modifications were made to the amino acid sequence to enhance brightness as reported by Ward and Chalfie in PCT publication WO 95/21191. Tsien, as disclosed in U.S. Pat. Nos. 5,625,048 and 5,777,079, provided modified forms of this protein which exhibited differing spectral characteristics and provided fluorescence of various colors. In addition, modifications were made to the nucleotide sequence encoding these proteins to make the sequence compatible with human cells. In addition, PCT publication WO 99/49019 published Sep. 30, 1999 provides some sequence information regarding green **fluorescent protein** expressed from genes isolated from *Renilla* and *Ptilocarpus*. Gurskaya, N. G., et al., BMC Biochem (2002) 2:5 describes mutations which change the spectrum of emission of **fluorescent protein** from coral.

Summary of Invention Paragraph - BSTX:

[0004] The above documents, each of which is incorporated herein by reference in its entirety, demonstrate that variations in the amino acid sequence of a **protein which exhibits fluorescence** upon excitation with radiation of shorter wavelength than the fluorescent wavelength provide a range of color choice and intensity. The **fluorescent proteins** have found wide use both in scientific research and in the production of **novelty items**, such as toys. Because the only requirements for fluorescence are irradiation with a suitable wavelength and because the **fluorescence is visible to the naked eye, these proteins** have proved convenient markers and have inspired whimsical applications.

Summary of Invention Paragraph - BSTX:

[0005] It has now been found that additional variants of **fluorescent protein** have improved brightness and exhibit low toxicity. These proteins can also be modified to fluoresce in a variety of colors and to vary in intensity.

Summary of Invention Paragraph - BSTX:

[0006] The invention is directed to compositions and methods which employ **fluorescent proteins** that are related by homology to the protein encoded by the nucleotide sequence set forth in FIG. 1B. The exemplified GFP contains, in

comparison to known fluorescent proteins, conservative substitutions and several substitutions which render it less acidic at the N-terminal portion but more acidic at the C-terminal portion. The invention is directed to compositions and methods related to a group of variants which are slightly less acidic in the N-terminal approximately half of the sequence and slightly more acidic at the C-terminal approximately half of the sequence. The proteins of the invention are non-toxic, and cells containing them can survive for at least 4 weeks and for 3, 4 or 6 months.

Brief Description of Drawings Paragraph - DRTX:

[0010] FIG. 3 shows a comparison of the amino acid sequence of the invention variant labeled A/C with a green fluorescent protein whose gene was cloned from R. mulleri.

Brief Description of Drawings Paragraph - DRTX

[0012] FIGS. 5A-5C show images of the fluorescent protein expressed in the packaging cell line PT67, the melanoma cell line B16F0, and the prostate cancer cell line PC3.

Brief Description of Drawings Paragraph - DRTX

[0014] FIGS. 7A and 7B show the nucleotide sequence encoding a fluorescent protein from coral, at positions 289-964.

Detail Description Paragraph - DETX:

[0015] Improved "green fluorescent proteins" (GFP) and recombinant materials which encode them are provided. This permits the use of a bright, nontoxic label to monitor gene expression, to label various cells, and to monitor the progress of metastases as described, for example, in PCT publication WO 98/49336, incorporated herein by reference. The improved fluorescent proteins of the invention offer more sensitive methods to assess these phenomena while remaining nontoxic to cells and entire organisms. Thus, the proteins of the present invention is useful in a variety of art known methods which employ the known forms of GFP described above. Production of GFP in general and use of recombinant materials as well as the GFP itself are well known in the art in view of the extensive literature describing the previously known forms of this fluorescent protein.

Detail Description Paragraph - DETX:

[0016] A protein of the amino acid sequence shown in FIG. 1A, designated A/C herein, emits green fluorescence and has the brightness and nontoxic properties stated above. However, as is known in the art, these "green" fluorescent proteins may be modified so that they fluoresce in various colors in the



visible spectrum. Thus, by suitably modifying the amino acid sequence of the protein set forth in FIG. 1A, red, yellow, blue, or other color fluorescence may also be obtained. In addition, the brightness of the fluorescence can be varied by making small changes to the amino acid sequence. The nature of such modifications is helpfully described in, for example, U.S. Pat. No. 5,777,079 incorporated herein by reference above. As described, modifications to the serine residue which is found at position 66 of the A/C sequence can be replaced by alanine, leucine, cysteine, valine, isoleucine or threonine to obtain proteins with red shifted spectra which are generally brighter as compared to the unmodified form of A/C. Other modifications that appear to affect brightness or fluorescence wavelength include those at position 67. The chromophore appears to be focused on positions 66-68 of the A/C protein, which correspond to positions 65-67 of the Aequorea wildtype GFP protein discussed in the '079 patent. Thus, mutations at positions 66-68 are particularly important in modifying the properties of the protein.

Detail Description Paragraph - DETX:

[0018] Additional modifications of 1-4 amino acids elsewhere in the molecule are also permitted; a minimal number of mutations of this type is insufficient to convert the A/C amino acid sequence into that of any known "green **fluorescent protein**" and has minimal impact on the properties of the molecule. Thus, the **proteins of the invention include fluorescent proteins** which are at least 90% homologous, preferably 95% homologous, and more preferably 99% homologous to the amino acid sequence shown in FIG. 1A. Particularly preferred are **fluorescent proteins** having the amino acid sequence shown in FIG. 1A and variants thereof which have at least one amino acid substitution in positions 66-68, preferably in position 66. Also preferred are modifications analogous to those described for the proteins from coral, described by Gurskaya, cited above.

Detail Description Paragraph - DETX:

[0019] The nucleotide sequence encoding the **fluorescent protein** variants of the invention may be expressed in a wide variety of cells. Expression systems suitable for production of proteins from recombinant systems are by now conventional for prokaryotes, eukaryotes such as yeast and fungi, higher plants, animal cells, including vertebrate cells, mammalian cells, and especially human cells, and a variety of cell lines. The appropriate expression system and vector depends on the nature of the host and the application intended. In addition to providing an appropriate expression system, the nucleotide sequence may be modified to convert it to a preferred codon usage for the intended host. The nucleotide sequence shown in FIG. 1B, thus, may contain one or more of the modifications shown in FIGS. 2A, 2B and 2C for expression in the indicated hosts, *Saccharomyces cerevisiae*, *Escherichia coli* and *Bifidobacterium longum*, respectively. Thus, the sequence for expression in *Saccharomyces cerevisiae* may contain 1-230 silent base changes; that for *E. coli* from 1-94 silent base changes and that for *Bifidobacterium longum* 1-21 base changes. All intermediate numbers of base changes are also included within the scope of the invention.

Detail Description Paragraph - DETX:

[0020] The fluorescent proteins of the invention and the recombinant materials encoding them may be applied in a wide variety of uses as is set forth in detail in PCT publication WO 99/49019, cited above, and incorporated herein by reference. This publication describes a many uses, including analytical, research, diagnostic, and commercial uses.

Detail Description Paragraph - DETX:

[0021] Thus, the object of the production of the fluorescent proteins of the invention may be to obtain the protein itself for use in various compositions and articles of manufacture. These fluorescent proteins may be used in various items such as toys, dolls, card games, paints, textiles, balloons, cosmetics, and foodstuffs or any other article or composition designed to glow. The protein itself may be produced for incorporation into these articles and compositions. The fluorescent proteins of the invention may also be combined with other materials which fluoresce or emit light, such as luciferase. The '019 publication describes compositions in which other luminescent biological materials are combined in the same composition with fluorescent proteins so that rather than effecting excitation by irradiation from an external source, the irradiating wavelengths are generated in situ by the luminescent combined material.

Detail Description Paragraph - DETX:

[0022] More serious uses of the green fluorescent protein focus on its value as a research tool. In one embodiment, the fluorescent proteins of the invention may be fused or otherwise coupled to antibodies directed to target tissues in plants or animals. For example, it may be desirable to label tumors in animals and to follow metastases by coupling the fluorescent label to the tumor. The fluorescent proteins of the invention may be prepared as conjugates with moieties which are able to target tissues or cells. Typical targeting moieties are specific binding partners for a material displayed on tissues or cells. Typical targeting moieties are antibodies and ligands for receptors.

Detail Description Paragraph - DETX:

[0023] Alternatively, the production of a fusion protein containing a green fluorescent protein can be used to monitor expression of the coupled protein. In addition, as described, for example, by Yang, M. et al., Cancer Res. (1998) 58:4217-4221 and by Yang, M. et al., Cancer Res. (1999) 59:731-736 and as reviewed by Hoffman, R. M., Cancer & Metastasis Reviews (1999) 17:271-277, fluorescent proteins may be generated in tumors and used to monitor metastasis.

Detail Description Paragraph - DETX:

[0024] The **fluorescent proteins** of the invention may also be used to label reagents in assays such as immunoassays. In one example, a sandwich assay may be employed wherein one specific binding partner to an analyte is a capture moiety which immobilizes the analyte and a second specific binding partner is used to label the immobilized analyte. The **fluorescent proteins** of the invention may be used directly as a label on the labeling binding partner or on a secondary binding partner such as, for example, the use of a second antibody-bearing label to couple to a first antibody directly bound to analyte.

Detail Description Paragraph - DETX:

[0025] Expression of a protein can also be monitored by fusing the nucleotide sequence encoding the **protein to a nucleotide sequence encoding the fluorescent protein** of the invention. Expression of the protein of interest may then be determined by monitoring the **fluorescence generated as the fusion protein** is produced. Alternatively, the capacity of a promoter or other control sequence to effect expression can be monitored by placing a nucleotide sequence encoding the invention **fluorescent protein** in operable linkage therewith. Again, fluorescence is generated by virtue of production of the **fluorescent protein**, thereby indicating that the expression controls are operable.

Detail Description Paragraph - DETX:

[0026] The foregoing applications are merely exemplary. In general, the **fluorescent proteins** of the invention can be used in any application where fluorescent labels are employed, including assay modifications such as fluorescence polarization and fluorescence quenching assays. The **fluorescent proteins** of the invention have the additional advantage of the capability of being generated in situ so that their presence or absence or amount can be used as an index of expression as well as to provide an internal source of fluorescence in cells. Thus, the course of tumor metastasis, bacterial or viral infection, or the movement of cells of any type within a plant or animal organism can be traced using the **fluorescent proteins** of the invention.

Detail Description Paragraph - DETX:

Expression of Improved **Fluorescent Protein**

Detail Description Paragraph - DETX:

[0028] A nucleic acid molecule encoding the **fluorescent protein** having the amino acid sequence shown as A/C in FIG. 1A was obtained, labeled R. reniformis GFP, from Stratagene (San Diego, Calif.). The nucleotide sequence encoding the protein was determined and is shown in FIG. 1B.

Detail Description Paragraph - DETX:

[0030] The amino acid sequence of the **fluorescent protein, in comparison to the fluorescent protein** in the art to which applicants believe it is most closely related, is shown in FIG. 3. The A/C protein is approximately 86% identical to the amino acid sequence of a protein encoded by a nucleotide sequence isolated from R. mulleri. The chromophore portions of these proteins, positions 66-68 of A/C and positions 65-67 of R. mulleri, are identical.

Detail Description Paragraph - DETX:

Preparation of Cell Lines Expressing A/C **Fluorescent Protein**

Claims Text - CLTX:

1. A **fluorescent protein** comprising the amino acid sequence of the A/C **protein of FIG. 1A or a fluorescent protein** having at least 90% homology with said A/C protein.

Claims Text - CLTX:

2. The **fluorescent protein** of claim 1, which has at least 95% homology with the A/C protein of FIG. 1A.

Claims Text - CLTX:

3. The **fluorescent protein** of claim 2, which has at least 99% homology with the A/C protein of FIG. 1A.

Claims Text - CLTX:

4. The **fluorescent protein** of claim 1, which comprises the amino acid sequence of the A/C protein of FIG. 1A.

Claims Text - CLTX:

5. The **fluorescent protein** of claim 1, wherein at least 1 amino acid in positions 66-68 has been replaced by a different amino acid.

Claims Text - CLTX:

6. The **fluorescent protein** of claim 5, wherein the amino acid at position 66 has been replaced.

Claims Text - CLTX:

7. A nucleic acid molecule comprising a nucleotide sequence which encodes the **fluorescent protein** of claim 1.

Claims Text - CLTX:

8. A nucleic acid molecule comprising a nucleotide sequence which encodes the **fluorescent protein** of claim 4.

Claims Text - CLTX:

10. A recombinant expression system which comprises a nucleotide sequence encoding the **fluorescent protein** of claim 1 operably linked to control sequences for its expression.

Claims Text - CLTX:

12. A method to produce a **fluorescent protein**, which method comprises culturing the cells of claim 11, wherein said nucleotide sequence is expressed to produce said **fluorescent protein, and optionally recovering said fluorescent protein.**

Claims Text - CLTX:

13. Antibodies specifically immunoreactive with the **fluorescent protein** of claim 1.

Claims Text - CLTX:

16. The expression system of claim 10, wherein said nucleotide sequence encoding the **fluorescent protein** is fused to a nucleotide sequence encoding additional protein.

Claims Text - CLTX:

17. A method to monitor the production of a protein, which method comprises providing an expression system which comprises a first nucleotide sequence encoding said protein fused to a second nucleotide sequence encoding the **fluorescent protein** of claim 1; placing said expression system in an environment in which expression is to be monitored; and assessing generation of fluorescence, whereby generation of **fluorescence indicates expression of said protein.**

Claims Text - CLTX:

18. A method to evaluate the activity of a promoter, which method comprises

providing a nucleic acid which comprises said promoter operably linked to a nucleotide sequence encoding **fluorescent protein** of claim 1; placing said nucleic acid in an environment in which the activity of the promoter is to be evaluated; monitoring appearance and amount of fluorescence; wherein the appearance and amount of fluorescence indicates the activity of the promoter.

Claims Text - CLTX:

20. A method to assess the position and progression of tumors and their metastases, which method comprises modifying said tumors to express the **fluorescent protein of claim 1 and observing the position of fluorescence**.

Claims Text - CLTX:

21. An improved method to conduct an immunoassay, wherein said immunoassay comprises entrapping analyte or an analog thereof in a sandwich comprising a first specific binding partner for said analyte coupled to a solid support and a second specific binding partner for said analyte comprising a label wherein the improvement comprises employing as a label the **fluorescent protein** of claim 1.

PGPUB-DOCUMENT-NUMBER: 20010036073

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010036073 A1

TITLE: Carvable decorative gourd

PUBLICATION-DATE: November 1, 2001

INVENTOR-INFORMATION:

| NAME                 | CITY  | STATE | COUNTRY | RULE-47 |
|----------------------|-------|-------|---------|---------|
| Trease, Christine K. | Price | UT    | US      |         |

APPL-NO: 09/ 838616

DATE FILED: April 19, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60198429 20000419 US

US-CL-CURRENT: 362/154,362/122 ,362/124 ,362/808

ABSTRACT:

An artificial hollow carvable gourd shaped as a pumpkin, squash, or other fruit or vegetable, having an outer carvable shell, which encases fake "innards and seeds" made of edible candy, and including a prize, contained therein to provide a more realistic gourd container.

RELATED APPLICATIONS

[0001] This application is a continuation-in-part application of the provisional application entitled "Carvable Decorative Gourd", Serial No. 60/1908,429, filed Apr. 19, 2000,

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Summary of Invention Paragraph - BSTX:

[0007] Cited for general interest is Bryan, U.S. Pat. No. 5,876,995, which discloses bioluminescent novelty items, which can have a Halloween theme.

Detail Description Paragraph - DETX:

convertible into a toy or novelty item suitable for decorative holiday use or play.



US-PAT-NO: 6126870

DOCUMENT-IDENTIFIER: US 6126870 A

TITLE: Chemiluminescent labeling compounds

DATE-ISSUED: October 3, 2000

INVENTOR-INFORMATION:

| NAME                  | CITY   | STATE | ZIP CODE | COUNTRY |
|-----------------------|--------|-------|----------|---------|
| Akhavan-Tafti; Hashem | Howell | MI    | N/A      | N/A     |

APPL-NO: 09/ 099657

DATE FILED: June 17, 1998

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of applicants' co-pending U.S. application Ser. No. 08/927,381 filed on Sep. 12, 1997.

US-CL-CURRENT: 252/700; 435/4 ; 435/5 ; 435/6 ; 435/7.1 ; 544/212 ; 544/96 ; 546/102 ; 546/103 ; 546/104

ABSTRACT:

Chemiluminescent labeling compounds and chemiluminescent labeled conjugates are provided. The compounds comprise an acridan ring bearing an exocyclic double bond and further contain a labeling substituent which permits attachment to compounds of interest. The novel chemiluminescent compounds and labeled conjugates generated chemiluminescence rapidly after undergoing a reaction with an acid, an oxidant and a base. The compounds and conjugates are useful in assays of an analyte in a sample and in assays employing labeled specific binding pairs.

22 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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Brief Summary Text - BSTX:

While many large molecules are used as labels, including enzymes and the

number of labels which can be attached to the target species and having the tendency of depositing non specifically on supports and surfaces.

Brief Summary Text - BSTX:

The present invention relates generally to methods of generating chemiluminescence and compounds for use in these methods. The methods use acridan compounds and simple, inexpensive and readily available reagents for generating chemiluminescence therefrom. The light producing reaction can be used for a number of art-recognized purposes, including analytical methods of assay, signaling, emergency lighting and novelty items.

US-PAT-NO: 6017769

DOCUMENT-IDENTIFIER: US 6017769 A

TITLE: Non-enzymatic methods of generating chemiluminescence from acridan alkenes

DATE-ISSUED: January 25, 2000

INVENTOR-INFORMATION:

| NAME                  | CITY   | STATE | ZIP CODE | COUNTRY |
|-----------------------|--------|-------|----------|---------|
| Akhavan-Tafti; Hashem | Howell | MI    | N/A      | N/A     |

APPL-NO: 09/ 099656

DATE FILED: June 17, 1998

US-CL-CURRENT: 436/544; 435/26 ; 435/28 ; 435/6 ; 435/7.1 ; 435/968 ; 436/546 ; 436/800 ; 436/805

ABSTRACT:

Methods of generating chemiluminescence rapidly without the use of enzymes are provided. The methods utilize chemiluminescent compounds comprising an acridan ring bearing an exocyclic double bond in a reaction with an acid, an oxidant and a base. The chemiluminescent methods are useful in assays to detect the amount of an analyte in a sample. The chemiluminescent compounds may be supplied as a label on an analyte or a specific binding partner or may be encapsulated within a liposome or latex particle.

35 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Brief Summary Text - BSTX:

While many large molecules are used as labels, including enzymes and the **photoprotein** aequorin, their use suffers the disadvantage of limiting the number of labels which can be attached to the target species and having the tendency of depositing non-specifically on supports and surfaces.

Brief Summary Text - BSTX:

The present invention relates generally to methods of generating chemiluminescence and compounds for use in these methods. The methods use acridan alkenes and simple, inexpensive and readily available reagents for generating chemiluminescence therefrom. The light producing reaction can be used for a number of art-recognized purposes, including analytical methods of assay, signaling, emergency lighting and novelty items.

Other Reference Publication - OREF:

H. Akhavan-Tafti et al, Bioluminescence and Chemiluminescence, Molecular Reporting with Photons, Proceedings of the 9th Internat'l Symp. on Biolumin. and Chemilumin. held at Woods Hole, MA, Oct. 1996, pp. 311-314, 1997.

US-PAT-NO: 5931383

DOCUMENT-IDENTIFIER: US 5931383 A

TITLE: Self-illuminated drinking straw

DATE-ISSUED: August 3, 1999

INVENTOR-INFORMATION:

| NAME               | CITY         | STATE | ZIP CODE | COUNTRY |
|--------------------|--------------|-------|----------|---------|
| Palmer; William R. | Cameron Park | CA    | N/A      | N/A     |
| Palmer; Stephen L. | Cameron Park | CA    | N/A      | N/A     |

APPL-NO: 09/ 017992

DATE FILED: February 3, 1998

US-CL-CURRENT: 239/33

ABSTRACT:

The instant invention provides for illuminated drinking straws which employ chemiluminescent mixtures as lighting sources. The illuminated drinking straw may be used with either hot or cold beverage such as water, fruit juices, soft drinks, coffees and teas, milk products or alcoholic beverages. A new and exciting drinking straw for amusement purposes is intended.

24 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

----- KWIC -----

Brief Summary Text - BSTX:

Other non-incandescent, chemical means of producing light which may be advantageously employed include **bioluminescent** systems, or alternately, chemiluminescent systems based on dioxetanes or other chemiluminescent reagents. Toy and novelty applications which utilizes **bioluminescent** systems are taught in PCT-WO 97/29319.

Brief Summary Text - BSTX:

The instant invention is directed to the use of a chemiluminescent device in

chemiluminescent lighting devices are enhanced by the inherent optical properties of beverages. Beverage fluid motion, color, clarity and degree of effervescence, if any, all serve to add to the interest of the instant invention. While chemiluminescence has been employed to produce various forms of illuminated drinking vessels and novelty items such as "swizzle" sticks, heretofore no device has been produced which utilizes the intrinsically interesting nature of beverage fluid travel in transparent or partially transparent tubes or drinking straws.

#### Brief Summary Text - BSTX:

For example, if the chemiluminescent device is producing a generally green or yellow light and a red beverage is drawn up through the device, the red beverage can filter out certain spectral portions of the chemiluminescent light to produce an apparent color change. Some dyes or coloring agents can be used not only as color filters but as fluorescers. A fluorescent dye functions by converting light of one wavelength to another wavelength. For example, blue light from a chemiluminescent device might be converted to red light by employing an appropriate fluorescer. This red light could be produced even if there was little or no red light emitted by the chemiluminescent device. U.S. Pat. No. 4,379,320 teaches to the use of secondary fluorescers similar to those described above. Of course, if such dyes or fluorescers were to be incorporated into a beverage it is necessary that they be completely safe for consumption. A variety of fluorescent proteins exist which may be used in this application, the use of said proteins being taught in PCT-WO 97/29319.

US-PAT-NO: 5858693

DOCUMENT-IDENTIFIER: US 5858693 A

TITLE: Device and method for phage-based antibiotic susceptibility testing

DATE-ISSUED: January 12, 1999

INVENTOR-INFORMATION:

| NAME                | CITY     | STATE | ZIP CODE | COUNTRY |
|---------------------|----------|-------|----------|---------|
| Cottingham; Hugh V. | Caldwell | NJ    | N/A      | N/A     |

APPL-NO: 08/ 883722

DATE FILED: June 27, 1997

PARENT-CASE:

This is a division of application Ser. No. 08/480,807, filed Jun. 7, 1995 now abandoned.

US-CL-CURRENT: 435/8; 435/32 ; 435/4 ; 435/5 ; 436/149 ; 436/63

ABSTRACT:

A phage-based antibiotic susceptibility test is carried out by maintaining a patient sample in a sealed sample well during addition of the phage and Luciferin substrate used in the test, in order to prevent contamination of the laboratory environment. The phage is adhered in dried form to a metal carrier disk which is retained beneath the cap of the sealed sample well by means of an external magnet, and is mixed with the patient sample by removing the external magnet and allowing the carrier disk to fall to the bottom of the sample well. The Luciferin substrate is adhered to the underside of the cap and is mixed with the patient sample by shaking or inverting the sealed sample well after the metal carrier disk has separated from the underside of the cap. A row of connected sample wells and caps may be employed to allow the same patient sample to be tested with multiple antibiotics.

3 Claims. 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets. 10

----- KWIC -----

Brief Summary Text - BSTX

While culture methods are useful in determining which antibiotic will be effective, they are very time-consuming, requiring as much as twelve weeks to determine antibiotic susceptibility. The resulting delay in beginning treatment can allow the disease to progress further, sometimes to the point where the patient dies. Recently, a new method has been developed which reduces the time necessary to determine antibiotic susceptibility to as little as two days. This method, which is disclosed in U.S. Pat. No. 4,861,709 to Ulitzur et al incorporated herein by reference, uses a specific bacteriophage which has the ability to infect the disease-causing bacteria. The phage causes the infected bacteria to produce an enzyme known as Luciferase. Luciferase is a well-known enzyme which, when combined with the substrate Luciferin, causes the substrate to emit light. In a phage-based test for antibiotic susceptibility, a patient sample is cultured individually with each antibiotic for a day or two. The phage is then added to the sample and incubated for a few hours, after which the Luciferin substrate is added. The sample is then observed for the presence of luminescence. If luminescence is present, the bacteria are still alive and the antibiotic with which they were initially cultured was not effective against them. If there is no observed luminescence, the bacteria are dead and the antibiotic with which they were initially cultured was effective against them.

#### Detailed Description Text - DETX:

The flexible magnetic strip 24 is preferably of the well-known type that is often used for so-called "refrigerator magnets" and similar types of novelty items. Extruded flexible magnetic strips of this type are available from Master Magnetics, Inc. of Castle Rock, Colo. as Product No. ZG-38. The flexibility of the magnetic strip 24, while not essential, allows it to be removed more easily from the cap strip 22 during the antibiotic susceptibility test. The metal carrier disks 26 may be made of any ferromagnetic metal, such as steel, and may consist of composite structures rather than solid metal. Examples of such composite structures include metal-coated plastic disks, plastic disks with embedded metal bodies or particles, and so on. It will also be appreciated that roles of the flexible magnetic strip 24 and metal carrier disks 26 may be interchanged, that is, the metal carrier disks 26 may be replaced with magnets and the flexible magnetic strip 24 may be replaced with a flexible metal or composite strip. As a further modification, the strip 24 and carrier disks 26 may both comprise magnets, with opposite poles positioned adjacent to each other. The top surface of the flexible strip 24 may be imprinted with a company logo or product name, instructions for use of the apparatus 10, or other printed information, either on the strip 24 directly or on a separate layer (not shown) adhered to its upper surface.

#### Detailed Description Text - DETX:

Following the phage incubation period, the Luciferin substrate 40 that is adhered to the underside of the caps 28 is mixed with the liquid biological sample 50. This is accomplished by either shaking or inverting the apparatus 10, or both, to bring the liquid biological samples 50 into contact with the dried Luciferin substrate 40. This causes the liquid biological samples 50 to



shown in FIG. 7C. The metal carrier disks 26, which are now free to move within the sample wells 12, serve as agitators to promote mixing between the samples 50 and the Luciferin substrate 40 during shaking or inversion of the apparatus 10. In FIG. 7D, a detection step is carried out by detecting any luminescence in the liquid biological samples caused by the combination of Luciferase (produced by live bacteria) with the Luciferin substrate. In order to avoid the need to open or unseal the sample wells 12 during the detection step, the caps 28 and cap strip 22 are preferably made either transparent or translucent, as noted earlier, so that any luminescence produced by the liquid biological samples 50 can be detected from the top of the sealed assembly 10. An automated instrument such as a luminometer is preferably used in the detection step, but the detection step can also be carried out manually if desired. In the example shown in FIG. 7D, the luminescence produced by the rightmost sample well 12 indicates that the bacteria in the liquid biological sample 50 are still alive, and hence that the antibiotic used in that sample well 12 was not effective to kill the bacteria. The lack of luminescence in the adjacent sample well 12 indicates that the bacteria in that sample well 12 are no longer viable, and hence that the antibiotic used in that sample well is effective against the particular bacterium in the patient sample. Similar results (i.e., either luminescence or non-luminescence) will be produced by the remaining sample wells 12 of the apparatus 10.

Claims Text - CLTX:

said reagent comprises a bacteriophage which induces luciferase production by said bacterium; and

Claims Text - CLTX:

said second reagent comprises luciferase.

US-PAT-NO: 5840963

DOCUMENT-IDENTIFIER: US 5840963 A

TITLE: Chemiluminescent reactions using dihydroxyaromatic compounds and heterocyclic enol phosphates

DATE-ISSUED: November 24, 1998

INVENTOR-INFORMATION:

| NAME                  | CITY     | STATE | ZIP CODE | COUNTRY |
|-----------------------|----------|-------|----------|---------|
| Akhavan-Tafti; Hashem | Brighton | MI    | N/A      | N/A     |

APPL-NO: / 021322

DATE FILED: February 10, 1998

PARENT-CASE:

This application is a divisional application of copending allowed application Ser. No. 08/855,421, May 13, 1997.

US-CL-CURRENT: 562/23; 562/25

ABSTRACT:

Novel methods and compositions which generate chemiluminescence are provided. The compositions comprise a heterocyclic enol phosphate compound and a dihydroxyaromatic compound in which the two hydroxy groups are separated by an even number of ring carbon atoms. Novel methods and compositions for generating chemiluminescence by reaction with a hydrolytic enzyme are provided as well. The compositions comprise a heterocyclic enol phosphate compound and a protected dihydroxyaromatic compound in which one of the hydroxy groups of the dihydroxyaromatic compound is protected with an enzyme-cleavable group. The novel chemiluminescent compositions are useful in methods for producing chemiluminescence for use in assays of hydrolytic enzymes and enzyme inhibitors and in assays employing labeled specific binding pairs.

3 Claims. 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Brief Summary Text - BSTX:

d. Luciferin Derivatives. Phosphate and galactoside derivatives of firefly luciferin are known (N. Ugarova, Y. Vosny, G. Kutuzova, I. Dementieva, Biolum. and Chemilum. New Perspectives, P. Stanley and L. J. Kricka, eds., Wiley, Chichester, 511-4 (1981); W. Miska, R. Geiger, J. Biolumin. Chemilumin., 4, 119-28 (1989)). Treatment of the firefly luciferin derivative with the appropriate enzyme liberates firefly luciferin which is reacted in a second step with luciferase and ATP to produce light.

Detailed Description Text - DETX:

The chemiluminescent reactions of the present invention comprising the reaction of compounds of formula I and III in the presence of oxygen may find use as chemical light sources, an example of which is the familiar light stick and related novelty items or for emergency lighting. Another use is in methods of detecting a compound of formula I in a sample in biomedical analysis, food analysis or environmental analysis of pollutants.

US-PAT-NO: 5772926

DOCUMENT-IDENTIFIER: US 5772926 A

TITLE: Chemiluminescent reactions using dihydroxyaromatic compounds and heterocyclic enol phosphates

DATE-ISSUED: June 30, 1998

INVENTOR-INFORMATION:

| NAME                  | CITY     | STATE | ZIP CODE | COUNTRY |
|-----------------------|----------|-------|----------|---------|
| Akhavan-Tafti; Hashem | Brighton | MI    | N/A      | N/A     |

APPL-NO: 08/ 855421

DATE FILED: May 13, 1997

US-CL-CURRENT: 252/700; 435/4

ABSTRACT:

Novel methods and compositions which generate chemiluminescence are provided. The compositions comprise a heterocyclic enol phosphate compound and a dihydroxyaromatic compound in which the two hydroxy groups are separated by an even number of ring carbon atoms.

Novel methods and compositions for generating chemiluminescence by reaction with a hydrolytic enzyme are provided as well. The compositions comprise a heterocyclic enol phosphate compound and a protected dihydroxyaromatic compound in which one of the hydroxy groups of the dihydroxyaromatic compound is protected with an enzyme-cleavable group.

The novel chemiluminescent compositions are useful in methods for producing chemiluminescence for use in assays of hydrolytic enzymes and enzyme inhibitors and in assays employing labeled specific binding pairs.

72 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

----- KWIC -----

Brief Summary Text - BSTX:

d Luciferin Derivatives Phosphate and galactoside derivatives of firefly

and Chemilum. New Perspectives, P. Stanley and L. J. Kricka, eds., Wiley, Chichester, 511-4 (1981); W. Miska, R. Geiger, J. Biolumin. Chemilumin., 4, 119-28 (1989)). Treatment of the firefly luciferin derivative with the appropriate enzyme liberates firefly luciferin which is reacted in a second step with luciferase and ATP to produce light.

Detailed Description Text - DETX:

The chemiluminescent reactions of the present invention comprising the reaction of compounds of formula I and III in the presence of oxygen may find use as chemical light sources, an example of which is the familiar light stick and related novelty items or for emergency lighting. Another use is in methods of detecting a compound of formula I in a sample in biomedical analysis, food analysis or environmental analysis of pollutants.

US-PAT-NO: 5554035

DOCUMENT-IDENTIFIER: US 5554035 A

TITLE: **Bioluminescent** algae in light bulb shaped viewing device

DATE-ISSUED: September 10, 1996

INVENTOR-INFORMATION:

| NAME          | CITY   | STATE | ZIP CODE | COUNTRY |
|---------------|--------|-------|----------|---------|
| Gooch, Van D. | Morris | MN    | 56267    | N/A     |

APPL-NO: 08/ 269696

DATE FILED: July 1, 1994

US-CL-CURRENT: 434/297; 119/245

ABSTRACT:

The present invention relates to an apparatus for viewing luminescence of algae. The apparatus comprises **bioluminescent**, dinoflagellate algae, an aqueous solution in which the **bioluminescent** algae can live, and a translucent light bulb shaped container for holding the **bioluminescent** algae in the aqueous solution.

8 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

TITLE - TI:

Bioluminescent algae in light bulb shaped viewing device

Abstract Text - ABTX:

The present invention relates to an apparatus for viewing luminescence of algae. The apparatus comprises **bioluminescent**, dinoflagellate algae, an aqueous solution in which the **bioluminescent** algae can live, and a translucent light bulb shaped container for holding the **bioluminescent** algae in the aqueous solution.

Brief Summary Text - BSTX:

The present invention relates generally to an educational and novelty device for viewing bioluminescent algae. In particular, the present invention relates to a light bulb shaped viewing device containing bioluminescent algae.

Brief Summary Text - BSTX:

One such scientific phenomenon that is particularly intriguing is luminescence. Prior art devices have attempted to encourage an interest in bioluminescence by placing bioluminescent algae in a clear cylindrical glass or plastic container to enable the bioluminescence to be viewed. The cylindrical container requires minimal care and provides a convenient means by which people can view bioluminescence.

Brief Summary Text - BSTX:

The present invention relates to an apparatus for viewing luminescence of algae. The apparatus comprises bioluminescent dinoflagellate algae, an aqueous solution in which the bioluminescent algae can live, and a translucent light bulb shaped container for holding the bioluminescent algae in the aqueous solution.

Detailed Description Text - DETX:

A device according to the present invention includes a light bulb shaped container containing bioluminescent dinoflagellate algae and an aqueous solution. The device provides a simple and educational mechanism for viewing bioluminescence.

Detailed Description Text - DETX:

By placing bioluminescent algae in a light bulb shaped container, children and adults are given the opportunity to view one of nature's more intriguing processes. Similar to a conventional light bulb, which emits light when turned on, the bioluminescent algae in the light bulb shaped container emit light when shaken. Because of the resemblance of the light bulb shaped bioluminescence viewing device to a light bulb, the device creates a greater interest in luminescence than merely placing the bioluminescent algae in a cylindrical container. As a result of the greater interest in luminescence, the device of the present invention stimulates a greater interest in science.

Detailed Description Text - DETX:

The phenomenon of bioluminescence is not a phenomenon that people commonly see. Certainly most people are rarely able to produce bioluminescence at will in

photosynthetically grow marine algae in one's home with minimal care. Hopefully, the ability to grow algae and view the luminescence will encourage people to better appreciate the importance of the wide diversity of organisms on our planet. It is also hoped that the device of the present invention stimulates curiosity and awe and the realization that there must be many more things that we do not yet fully understand.

#### Detailed Description Text - DETX:

The **bioluminescent** algae selected for use in the device of the present invention display a bright luminescence in response to agitation. The **bioluminescent** algae are also sufficiently hardy so that a significant proportion of the algae remain alive while the device is handled prior to and after sale to a consumer. There are several genera of **bioluminescent**, photosynthetic, dinoflagellate algae that are suitable for use with the present invention. Preferably, the genera include Pyrocystis, Dissodinium, Noctiluca, Gonyaulax, Peridinium, Pyrodinium, and Ceratium. The preferred species that exhibits the best balance between luminescence and durability is Pyrocystis lunula. This species has also been known as Dissodinium lunula.

#### Detailed Description Text - DETX:

Because the **bioluminescent** algae are photosynthetic, they need light to carry out photosynthesis. While the algae can survive for 4-7 days without light, at least 5 hours per day of indirect light from a window or light from a 40 Watt fluorescent bulb is needed for good survival of Pyrocystis.

#### Detailed Description Text - DETX:

The algae placed in the light bulb shaped container only luminesce when the surroundings are dark. Once the surroundings are changed from dark to light, the ability of the algae to luminesce drops significantly. The process of changing the surroundings from dark to light is referred to as photoinhibition. The amount of the photoinhibition depends on the intensity of the light. For example, in the presence of a fluorescent light the ability to luminesce drops to about half in 5 minutes and drops to 10% in about 15 minutes for Pyrocystis as described by W. H. Biggley et al., Stimulable and Spontaneous **Bioluminescence** in the Marine Dinoflagellates, Pyrodinium bahamense, gonyaulax polydera, and Pyrocystis lunula, 54 J. GEN. PHYSIOLOGY 96-122 (1972). After being fully photoinhibited, the algae can recover by placing them back into the dark. To achieve 50% recovery takes about 5 minutes and 90% recovery takes 15 minutes.

#### Detailed Description Text - DETX:

Another issue that must be addressed when selecting algae for use in the device of the present invention is when the luminescence can be viewed. Some of the dinoflagellates show strong internal daily cycles called circadian rhythms. As



even when placed in the dark if it is during the normal day phase of their cycle. The Pyrocystis are preferable for use in the present invention because the Pyrocystis only weakly shows circadian rhythms as described by Elijah Swift & Valerie Meunier, Effects of Light Intensity on Division Rate, Stimulable **Bioluminescence** and Cell Size of the Oceanic Dinoflagellates Dissodinium Lunula, Pyrocystis Fusiformis, and P. Noctiluca, 12 J. PHYCOLOOY 14-22 (1976). On the other hand, Gonyaulax are less preferably because the Gonyaulax exhibits strong circadian rhythms.

#### Detailed Description Text - DETX:

For most types of algae, the **bioluminescence** is only seen upon agitation of the organisms. While Pyrocystis are not detrimentally affected by agitation, agitation may be quite harmful to Gonyaulax. The algae can be repeatedly agitated to luminescence for about 5 minutes. After this point, the algae must be allowed to regenerate for a day cycle.

#### Detailed Description Text - DETX:

When selecting the **bioluminescent** algae for use in the device of the present invention, there is an additional concern that the **bioluminescent** algae must not be toxic to humans. Toxicity is especially important because the **bioluminescence** of the algae is particularly intriguing to young children.

#### Detailed Description Text - DETX:

Bioluminescent algae toxicity questions typically arise when there are large shell fish kills. The shell fish kills are frequently linked to shell fish consuming large amounts of certain species of bioluminescent **dinoflagellates** and concentrating certain chemicals from those algae.

#### Detailed Description Text - DETX:

The preferred algae, Pyrocystis, used in the present invention have never been implicated as one of the dinoflagellates involved in shell fish poisoning as described by Karen A. Steidinger & Daniel G. Baden, Toxic Marine Dinoflagellates, in DINOFLAGELLATES 201-61 (David Spector, ed., 1984). If one drank a culture of the dinoflagellates from the device of the present invention, it is highly unlikely that there would be a sufficiently high concentration of toxins to have a detrimental effect. For purposes of incorporating the **bioluminescent** algae into a **novelty item**, no species that was known to be involved in shell fish poisoning is used.

#### Detailed Description Text - DETX:

The algae and the aqueous solution are then placed in the bulb 12. The algae and the aqueous solution preferably fill approximately 2/3 times of the bulb

bulb 12, the cap 14 is screwed on the bulb 12. The cap 14 tightly engages the bulb 12 so as to prevent leakage of the algae and the aqueous solution from the container 10.

Claims Text - CLTX:

1. An apparatus for viewing luminescence of algae, the apparatus comprising: **bioluminescent**, dinoflagellate algae;

Claims Text - CLTX

an aqueous solution in which the **bioluminescent** algae can live; and

Claims Text - CLTX

a light bulb shaped container having a bulbous portion and a neck portion extending from the bulbous portion, the light bulb shaped container holding the **bioluminescent** algae and the aqueous solution, at least a portion of the light bulb shaped container being translucent, where in the aqueous solution has the following composition:

Claims Text - CLTX

2. The apparatus of claim 1 wherein the **bioluminescent**, dinoflagellate algae are selected from the group consisting of the following genera: Pyrocystis, Dissodinium, Noctiluca, Gonyaulax, Peridinium, Pyrodinium, and Ceratium.

Other Reference Publication - OREF:

Elijah Swift & Valerie Meunier, Effects of Light Intensity on Division Rate, Stimulable **Bioluminescence** and Cell Size of the Oceanic Dinoflagellates Dissodinium lunula, Pyrocystis fusiformis, and P. noctiluca, 12 J. Phycology 14-22 (1976).

Other Reference Publication - OREF:

W. H. Biggley et al., Stimulable and Spontaneous **Bioluminescence** in the Marine Dinoflagellates, Pyrodinium bahamense, Gonyaulax polydera, and Pyrocystis lunula, 54 J. Gen. Physiology 96-122 (1972).